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Increased Echodensity of Myocardial Wall in the Diabetic Heart: An Ultrasound Tissue Characterization Study

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Objectives. **We sought to characterize myocardial echodensity in asymptomatic patients with insulin-dependent diabetes and normal conventional two-dimensional echocardiographic findings to determine whether ultrasound tissue characterization can detect ultrastructural changes in myocardium, such as an increase in collagen content.**

Background. **Fibrosis alters the acoustic properties of the heart in animals and humans, and these changes are detectable by cardiac tissue characterization with ultrasound. Early changes detected in the diabetic heart include increased interstitial collagen deposition.**

Methods. **Using two-dimensional echocardiography, we evaluated 26 asymptomatic patients with insulin-dependent diabetes with normal regional and global rest function, and 17 age- and gender-matched control subjects. By selection, all diabetic patients were normotensive and had negative maximal exercise stress test results to avoid the confounding effects of hypertension and coronary artery disease. Using an echocardiographic instrument implemented at the Institute of Clinical Physiology, we performed an on-line radiofrequency analysis to obtain quantitative operator-independent measurements of the integrated backscatter signal of the ventricular septum and posterior wall. The integrated values of the radiofrequency signal from the myocardial wall were normalized for those from the pericardial interface and were expressed as percentages (integrated backscatter index).**

Results. **Diabetic patients showed a significant increase in myocardial echodensity both in the septum ([mean ± SD] 36.6 ± 8.1 vs. 23.6 ± 4.4, $p < 0.0001$) and posterior wall (21.2 ± 5.3 vs. 18.4 ± 3.7, $p < 0.001$). By individual patient analysis, 17 patients exceeded the 95% confidence limits for normal myocardial echocardiographic reflectivity found in normal subjects, and only 3 had a relatively abnormal transmitral Doppler filling pattern (E/A ratio), mainly consisting of an abnormally increased late peak flow velocity (65% vs. 11%, $p < 0.001$). The increased myocardial intensity was similar in patients with ($n = 16$) and without ($n = 10$) noncardiac complications, such as retinopathy or nephropathy (37.5 ± 7.9% vs. 35.0 ± 8.3%, $p = 0.35$).**

Conclusions. **Abnormally increased myocardial echodensity, possibly related to collagen deposition, can be detected in asymptomatic diabetic patients with normal rest function. Theoretically, this finding might be considered a very early preclinical alteration potentially related to subsequent development of diabetic cardiomyopathy.**

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Ultrasound tissue characterization is a technique now available for clinical studies that can identify and characterize abnormalities in the physical or physiologic state of biologic structures on the basis of analyzing the interactions between ultrasound and tissue (1–4). In particular, ultrasound tissue characterization with backscatter analysis in the radiofrequency domain provides a particularly robust, but technically demanding, method of quantitatively assessing the acoustic properties of myocardium. Two main variables can be derived from backscatter analysis: 1) indexes for measuring cyclic (systolic to diastolic) variation that are mainly linked to intramural function and contractility, albeit in a complex, only partially understood manner (1–4), and 2) indexes for measuring the absolute echodensity value that are mainly linked to the structural, histologic component of the myocardial tissue, such as collagen content (1–4). This technique has been applied in several myocardial diseases and shows potential for preclinical detection of myocardial involvement, even at a stage when conventional echocardiographic indexes are within the normal range. This early involvement has been documented by ultrasound tissue characterization in a variety of pathologic conditions, including cardiac hemochromatosis (5), myocardial ischemia (6) and acute cardiac rejection (7,8). In the diabetic human heart, Perez et al. (9) have documented a blunted cyclic backscatter variation in the absence of changes in echocardiographically assessed structure and function. The behavior of absolute echodensity in the myocardial wall of diabetic heart remains unknown. The hypothesis underlying the present study is that a change in myocardial structure may occur in the early stage of the diabetic disease in the absence of any clinical sign of cardiac involvement (10). Experimental (11–13) and human

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Table 1. Demographic Features, Hemodynamic Factors and Metabolic Patterns

	Group A	Group B	p Value
Age (yr)	32.6 ± 10.0	32.6 ± 9.9	0.48
Gender (M/F)	10/16	7/10	
Height (cm)	166.5 ± 7.4	168.8 ± 9.5	0.23
Weight (kg)	62.7 ± 8.5	63.7 ± 10.7	0.65
Body surface (m^2)	1.72 ± 0.1	1.72 ± 0.2	0.50
SAP (mm Hg)	123.4 ± 12.7	119.7 ± 10.4	0.17
DAP (mm Hg)	75.6 ± 7.5	75.0 ± 6.1	0.93
MAP (mm Hg)	91.5 ± 8.7	89.9 ± 6.7	0.85
Heart rate (/min)	75.6 ± 10.1	79.6 ± 9.5	0.78
Serum cholesterol (mmol/liter)	4.72 ± 1.10	5.17 ± 1.14	0.67
HDL cholesterol (mmol/liter)	1.15 ± 0.21	1.19 ± 0.9	0.79
Triglycerides (mmol/liter)	1.00 ± 0.35	1.09 ± 0.64	0.34
Lipoprotein (a) (mg/dl)	17.4 ± 21.8	12.2 ± 14.8	0.54
Apolipoprotein A1 (mg/dl)	151.7 ± 25.7	150.6 ± 23.2	0.28
Apolipoprotein A2 (mg/dl)	87.4 ± 24.4	93.2 ± 19.6	0.56
HbA1c (%)	8.3 ± 1.5		
Serum urea (mmol/liter)	12.8 ± 3.5	13.3 ± 6.4	0.83
Serum creatinine (μmol/liter)	80.9 ± 12.9	89.3 ± 10.1	0.56
Serum glucose (mmol/liter)	12.2 ± 5.6	4.8 ± 0.6	< 0.0001
Urea clearance (ml/min)	41.5 ± 12.7	39.2 ± 10.9	0.73
Creatinine clearance (ml/min)	89.3 ± 26.4	90.6 ± 16.3	0.18
Urine glucose (mmol/24 h)	53.6 ± 69.8	0.35 ± 0.2	< 0.001
Serum albumin (g/liter)	44.8 ± 5.1	47.3 ± 6.0	0.23
Urine albumin (mg/24 h)	33.3 ± 44.1	28.3 ± 35.9	0.31

Data presented are mean value ± SD or number of patients or subjects. DAP = diastolic arterial pressure; F = female; Group A = diabetic patients; Group B = normal control subjects; HbA1c = glycosylated hemoglobin; HDL = high density lipoprotein; M = male; MAP = mean arterial pressure; SAP = systolic arterial pressure.

pathologic (14) evidence behind this hypothesis documents that diabetic hearts without significant coronary lesions show collagen accumulation in perivascular loci, between myofibers or as replacement fibrosis (10). The collagen accumulation is linearly related to ultrasound backscatter, as shown concordantly by experimental (1–4,15) and clinical (16,17) studies.

In the present study, we sought to assess in vivo myocardial tissue echoreflectivity in diabetic human hearts and to correlate this finding with clinical, metabolic, two-dimensional and Doppler echocardiographic data.

Methods

Study patients. Twenty-six patients with type I diabetes were recruited from the Diabetes Unit of the Second Medical Clinic of Pisa University. By selection all patients were asymptomatic, with normal findings on physical examination, negative maximal exercise stress test results and a technically good baseline echocardiographic study. A control group of 17 normal subjects was also evaluated. The two groups were comparable in age, weight and body surface. No subject had pathologic systolic and diastolic arterial pressures, defined according to World Health Organization criteria (18). A representative value of arterial blood pressure was measured by cuff sphygmomanometer at echocardiographic study. Mean arterial pressure (MAP) was calculated as follows: MAP = DAP + 1/3(SAP − DAP), where DAP (SAP) = diastolic (systolic) arterial pressure. The demographic features of the two groups are reported in Table 1. Exclusion criteria were the documentation of valvular heart disease by Doppler analysis, ventricular dyssynergies of contraction and patients who smoked >5 cigarettes/day.

Evaluation of metabolic and clinical variables. Serum cholesterol, triglyceride, urea, creatinine and glucose levels were determined with a BM/Hitachi System 717 model (Hitachi Ltd., Tokyo, Japan) and reagents from Boehringer Mannheim. Glycosylated hemoglobin was evaluated by the Biorad DIAMAT fully automated glycosylated hemoglobin Analyzer System. Urine urea, creatinine and glucose were assayed using a Synchron Cx3 (Beckman Instruments Inc.) automated analyzer. High density lipoprotein (HDL) cholesterol was measured after precipitation of other lipoproteins with phosphotungstic acid and reagents from Menarini (Florence, Italy). Lipoprotein (a) was measured with a commercial enzyme-linked immunosorbent assay [Immunoenzyme Lp (a)] (Immuno Division Diagnostic, Pisa, Italy). Apolipoproteins A1 and B were determined by the kinetic immunonephelometric method with an automated instrument (Bering Institute nephelometer and reagents). Serum and urine albumin were also determined by the immunonephelometric method. All diabetic subjects were taking insulin therapy (mean daily insulin dose 0.78 ± 0.12 IU/kg) (19). On the day before the examination, subjects collected a 24-h urine specimen to be analyzed for urinary albumin, creatinine, urea and glucose. After an over-

Figure 1. Top, Two-dimensional image showing left ventricular long-axis view, with the sample volume positioned on the septum strictly perpendicular to the ultrasound beam in a control subject. **Bottom**, Relative backscattered radiofrequency signal corresponding to the septal area.

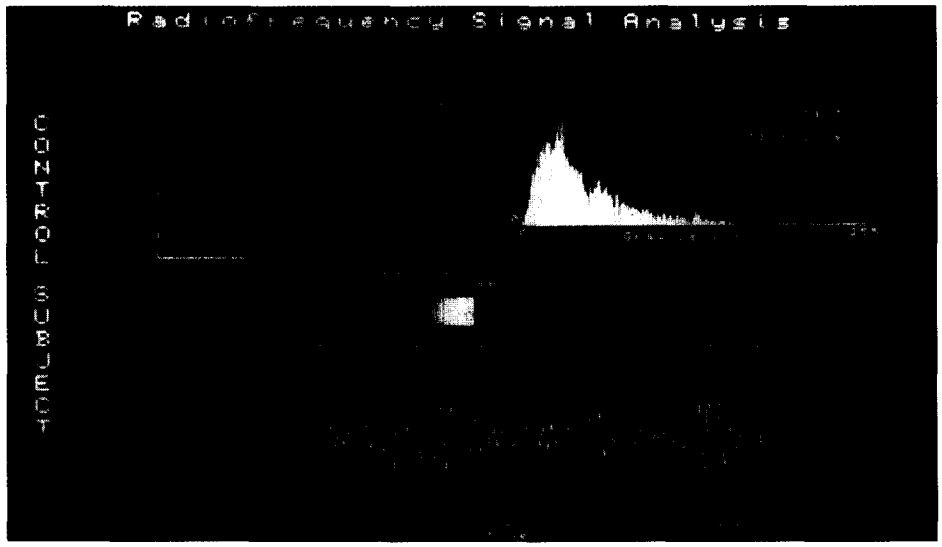
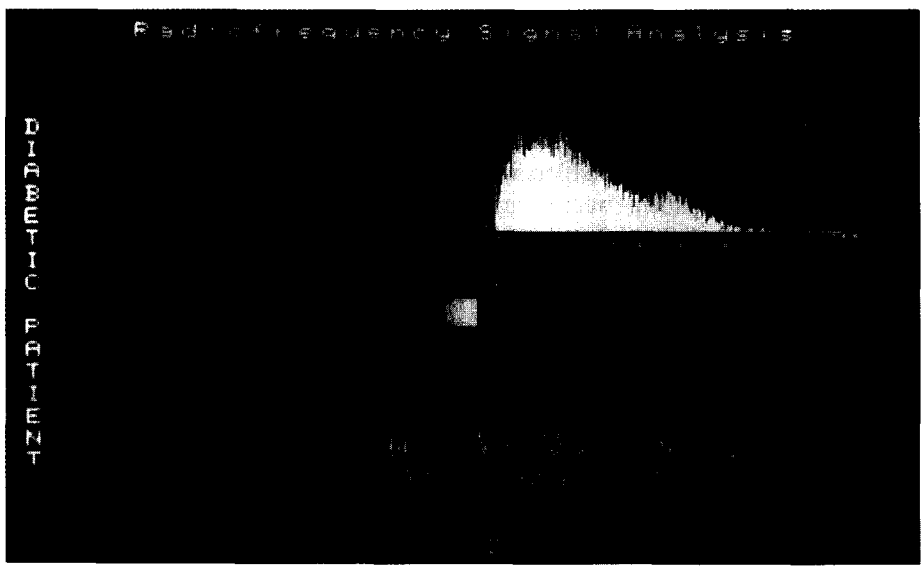


Figure 2. Top, Two-dimensional image showing left ventricular long-axis view, with the sample volume positioned on the septum strictly perpendicular to the ultrasound beam, in a diabetic patient. **Bottom**, Relative backscattered radiofrequency signal corresponding to the septal area.



night fast, venous blood was drawn for plasma glucose, creatinine, urea, glycosylated hemoglobin, total and HDL cholesterol and triglyceride analysis. All of these metabolic variables were analyzed in the same manner in the control group.

The tests used to evaluate autonomic neuropathy were the following: heart rate variability on six maximal breaths per min, heart rate increase on standing at 15 s, Valsalva ratio and postural systolic blood pressure decrease on standing (20).

Conventional Doppler echocardiography. M-mode and two-dimensional echocardiograms with Doppler analysis were obtained for all subjects by means of a commercially available machine (Hewlett-Packard Sonos 1000 with 2.5- or 3.5-MHz transducer). During the echocardiographic examination, all subjects were in the semisupine left lateral decubitus position. Two-dimensional images were obtained in the parasternal long- and short-axis views and in the apical four- and two-chamber views by using standard transducer positions. The following variables were measured from the M-mode echocardiographic tracings guided by two-dimensional imaging: 1) end-diastolic diameter (cm); 2) end-diastolic diameter index, as end-diastolic diameter/body surface ratio (cm/m^2); 3) percent fractional shortening of the left ventricle (%); 4) septal and posterior wall thickness at end-diastole (cm); and 5) left ventricular mass (g) by American Society of Echocardiography formula and left ventricular mass index (g/m^2) (left ventricular mass/body surface ratio) (21).

Pulsed Doppler transmitral flow velocity profile was obtained from the apical four-chamber view, and the sample volume was positioned just below the mitral valve leaflets. The following variables were evaluated: 1) peak E (peak transmitral flow velocity in early diastole); 2) peak A (peak transmitral flow velocity in late diastole); 3) E/A ratio; 4) mitral acceleration time (from baseline to peak E wave), corrected for heart rate; 5) mitral deceleration time (from peak E wave to baseline), corrected for heart rate, and 6) isovolumetric relaxation time (by placing the sample volume at an intermediate point between the mitral and aortic valves, it was measured as the interval from the end of the left ventricular outflow velocity to the onset of mitral inflow), corrected for heart rate. Measurements were derived from the average of at least five consecutive cardiac cycles. Mitral acceleration and deceleration times and isovolumetric relaxation time were corrected for heart rate by Bazett's formula ($time/\sqrt{RRinterval}$).

Ultrasound tissue characterization. An OTE Biomedica AU 530 two-dimensional mechanical sector scanner echocardiograph was used for spatial orientation of the ultrasound beam; quantitative analysis of ultrasound reflectivity was performed in the regions of interest, that is, the ventricular septum and the posterior left ventricular free wall. These regions were visualized from the parasternal long-axis view. The backscattered signal was acquired at end-diastole because a systematic variation in backscatter amplitude occurs during the cardiac cycle (1,2).

A 3.5-MHz frequency transducer was used (focal distance 7 cm, −3 dB, focal region 6 cm). The transducer bandwidth, measured at −3 dB with respect to the 3.5-MHz central frequency, was 700 kHz. The "native" (raw) radiofrequency signal was sampled before the processing chain of the two-dimensional instrument. Briefly, the radiofrequency signal undergoes preamplification, bypassing the receiving circuits of the ultrasound equipment. The analog signal is fed to an amplifier, and the gain sweep of the amplifier (from 2 to 60 dB) is accomplished in 30 steps. Such operation allows full use of the input dynamic range of the analog to digital converter. Sampling is performed by a flash converter with 8 bits of amplitude resolution, at a rate of 40 MHz. The digitized signal, from analog to digital converter, is analyzed in real time by a hardware prototype developed in our electronics laboratory. The acquisition of the two-dimensional gate is visualized on the two-dimensional image to ensure its proper positioning perpendicular on the selected beam (Fig. 1 and 2). For analysis of the myocardium, the gate width was kept at 3 μs, which corresponds to 2.35 mm (for 128 points), given the velocity of ultrasound in biologic tissues of 1.57 mm/μs. This allowed sampling on the radiofrequency signal in the middle layers of the myocardium, thus excluding epicardial and endocardial specular reflections. The acquisition gate was kept immediately behind the specular echo of the endocardium (left endocardium for the septum) to minimize the transmural variations in backscatter, which are influenced by the position from which the signal is acquired within the wall (Figs. 1 and 2). The representative value for the ventricular septum and posterior free wall was calculated as the average of three values. For evaluation of the pericardial echo, we used a 1.5-μs gate length (which corresponds to 1.2 mm, for ~64 points) (4). The acquisition gate was centered on the strongest pericardial reflections, just behind the mitral leaflets. The representative value for the pericardial echo was calculated as the mean of three values (mean variability 4.5%). Hardware analysis involved measurement of the integrated amplitude of the rectified radiofrequency signal corresponding to the two-dimensional area selected from the echocardiographic image. The system provided a simultaneous display of conventional information together with tissue characterization variables (the two-dimensional integrated backscatter alphanumeric index and the lateral displacement profile averaged over the selected depth). Alphanumeric two-dimensional integrated backscatter data values were transferred on-line to a personal computer (model AT) for statistical analysis. The reproducibility of the method was good: In our laboratories the coefficient of correlation for intraobserver measurements was $r = 0.92$; that for interobserver measurements $r = 0.88$.

Ultrasound quantitative data analysis. Primary, not normalized or compensated, two-dimensional integrated backscatter values were not considered for analysis because they are highly influenced by chest morphology, heart structure depth and ultrasound impedance, which differ patient by patient. Therefore, to assess quantitatively the reflectivity of the ventricular septum and posterior free wall, we used the percent two-dimensional integrated backscatter, expressed as a com-

Table 2. Conventional and Doppler Echocardiographic Variables

	Group A	Group B	p Value
EDD (cm)	4.43 ± 0.4	4.50 ± 0.3	0.23
EDD_i (cm/m^2)	2.57 ± 0.3	2.6 ± 0.19	0.34
FS (%)	40.1 ± 4.6	38.7 ± 3.8	0.51
STh (cm)	0.94 ± 0.09	0.96 ± 0.06	0.25
PWTh (cm)	0.95 ± 0.09	0.95 ± 0.08	0.51
LVM (g)	169.1 ± 46.2	169.0 ± 34.2	0.9
LVM_i (g/m^2)	97.3 ± 20.9	97.5 ± 12.7	0.75
Peak E (m/s)	0.88 ± 0.2	0.86 ± 0.1	0.34
Peak A (m/s)	0.69 ± 0.1	0.58 ± 0.1	< 0.01
E/A ratio	1.30 ± 0.3	1.55 ± 0.33	< 0.01
MATc	2.6 ± 0.5	2.6 ± 0.5	0.28
MDTc	4.8 ± 0.9	5.1 ± 0.7	0.23
IVRTc	3.5 ± 0.8	3.6 ± 0.3	0.42

Data presented are mean values ± SD. EDD = end-diastolic diameter; EDD_i = end-diastolic diameter index; FS = percent fractional shortening of left ventricle; Group A = diabetic subjects; Group B = normal control subjects; IVRTc = corrected isovolumetric relaxation time; LVM = left ventricular mass; LVM_i = left ventricular mass index; MATc = corrected mitral acceleration time; MDTc = corrected mitral deceleration time; Peak A = peak transmitral flow velocity in late diastole; Peak E = peak transmitral flow velocity in early diastole; PWTh = posterior wall thickness at end-diastole; STh = septal wall thickness at end-diastole.

parison with the pericardial interface. To obtain quantitative assessment of the reflectivity of the ventricular septum and posterior free wall, we used the percent two-dimensional integrated backscatter, expressed as a comparison with the pericardial interface. Two-dimensional integrated backscatter results for each heart structure, initially displayed in mV, were then expressed in percent values, assigning 100% to the pericardial interface (from which the maximal echointensity was consistently recorded in the individual patients). The individual pericardial signal strength was used to normalize myocardial signals in each patient.

Statistical analysis. Continuous variables were expressed as mean value ± 1 SD. Differences were tested for significance by paired and unpaired Student *t* tests. Upper and lower 95% confidence limits for each variable were calculated from the two tails of the Student *t* test distribution using the following formulas: mean value ± (2.042 × SD) and mean − (2.042 × SD), respectively. Relations between radiofrequency and two-dimensional echocardiographic measurements were expressed in terms of linear regression analysis; chi-square analysis was used to compare categorical variables; $p < 0.05$ was considered significant.

Results

Clinical, hemodynamic and metabolic data from the diabetic patients and normal control subjects are reported in Table 1. The two groups were similar for heart rate and blood pressure values, as well as for metabolic variables except for serum and urine glucose. Of the 26 type I diabetic patients, 10 had uncomplicated courses (mean diabetes duration 5.2 ± 2.5 years). Sixteen subjects (mean diabetes duration 17.2 ± 5.6 years) had noncardiac complications, consisting of retinopathy in 15 (background or proliferative retinopathy alone in normoalbuminuric normotensive diabetic patients) with or without "incipient nephropathy" in 7 (persistent microalbuminuria, urinary albumin excretion rate [22] >20 but <200 μg/min in diabetic patients with concomitant retinopathy but without any other renal or urinary tract disease). No diabetic patient had clinical or laboratory findings of autonomic neuropathy.

Table 3. Ultrasound Quantitative Data

Integrated Backscatter Index	Group A	Group B	p Value
Septum (%)	36.6 ± 8.1	23.6 ± 4.4	< 0.0001
Posterior wall (%)	21.2 ± 5.3	18.4 ± 3.7	< 0.001
Pericardium	103.9 ± 19.8	109.8 ± 5.6	0.173

Data presented are mean value ± SD. Group A = diabetic subjects; Group B = normal control subjects.

Technically satisfactory conventional echocardiographic images and radiofrequency ultrasound signals were obtained for each subject.

M-mode echocardiographic findings. M-mode echocardiographic measurements were similar in diabetic patients and control subjects and are reported in Table 2.

Doppler transmitral flow findings. Of the Doppler indexes evaluating left ventricular diastolic filling, late peak flow velocity was significantly higher in diabetic patients ($p < 0.01$); early peak flow velocity (E) was similar between the groups. The E/A ratio was significantly higher in the control group than in diabetic patients ($p < 0.01$; Table 2). When individual patient analysis was performed, the E/A ratio was beyond the 95% confidence limits, obtained from the normal control subjects, in 11% of the study patients.

Quantitative analysis of ultrasound backscatter. Table 3 reports the percent and compensated two-dimensional integrated backscatter values of the ventricular segments analyzed in diabetic patients and in the normal control subjects. The integrated backscatter index of the septum and left ventricular posterior wall was significantly higher in diabetic patients than that in the control group (Fig. 3). Among diabetic patients, no significant difference in integrated backscatter index in the septum was found between those with (n = 16) and without (n = 10) noncardiac complications (37.5 ± 7.9% vs. 35.0 ± 8.3%, p = 0.35).

Relation between conventional echocardiographic measurements and radiofrequency analysis. In diabetic patients, no significant correlation was found between regional myocardial reflectivity (considered as percent integrated backscatter) of the septal and posterior walls and the corresponding wall thickness ($r = -0.31$, $p = 0.28$ and $r = -0.09$, $p = 0.65$, respectively; Fig. 4). No significant correlation was found between the septal percent backscatter value and left ventricular end-diastolic diameter ($r = -0.04$, p = NS).

When individual patient analysis was performed, the septal backscatter index was beyond the 95% confidence limits

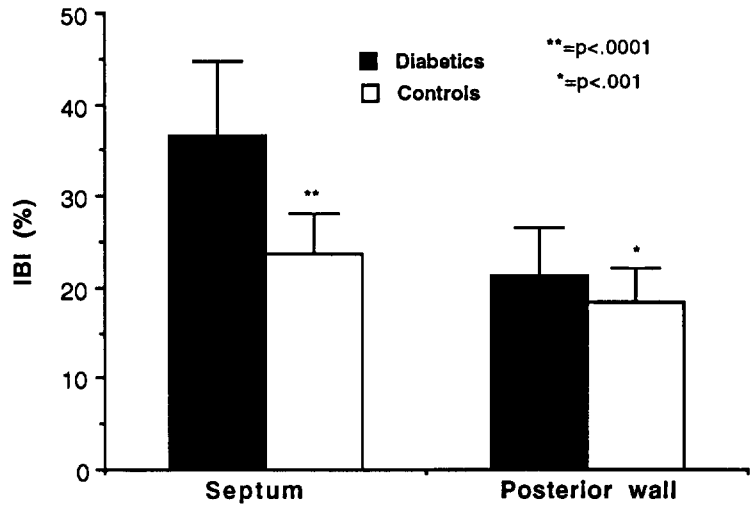


Figure 3. Percent two-dimensional integrated backscatter index (IBI) values of the septum and posterior wall in patients with diabetes (**solid bars**) and in control subjects (**open bars**). Quantitative reflectivity of the myocardial walls appears to be significantly higher in diabetic patients.

obtained from the normal group in 17 (65%) of the 26 study patients, significantly higher in comparison with those selected with Doppler analysis (11%; $p < 0.001$).

Discussion

Abnormally increased myocardial echodensity—possibly related to collagen deposition—can be detected in asymptomatic diabetic patients with normal rest function and may provide a very early and preclinical finding probably related to the development of a subsequent clinical "diabetic cardiomyopathy" in these patients. The increased myocardial echodensity was similar in patients with and without noncardiac complications. Septal backscatter measurements yielded higher values compared with posterior backscatter measurements in both control and diabetic subjects. This finding has been reported previously and consistently in normal subjects (23) and is probably due to the ultrasound beam attenuation and to the technical characteristics of the acquisition system used.

Biologic basis of increased myocardial acoustic reflectivity. The most likely explanation for the increased myocardial acoustic reflectivity of the diabetic heart is an augmentation of the connective tissue content of the myocardium. Some theoretic and experimental evidence shows that collagen is a primary determinant of echocardiographic scattering in myocardial tissue and that there is a linear relation between collagen deposition and backscatter magnitude (1–4,15). Several clinical studies (16,17) have confirmed a good correlation between the amplitude of the echo and histologically or biochemically assessed collagen content.

On the basis of published evidence, the increased reflectivity in the walls of the diabetic heart should be paralleled by a similar increase in connective tissue deposition. Some observations support this hypothesis, derived from experimental, autopsy and epidemiologic studies. Some experimental observations (24) in rats with induced diabetes showed an increase in intercellular and perivascular deposition of connective tissue. In postmortem examinations, the most frequent histopathologic findings in diabetic subjects with clinical evidence of congestive heart failure are a variable degree of interstitial and perivascular fibrosis and subendocardial thickening of the small coronary arteries (25); in addition, a subgroup of diabetic patients without clinical evidence of heart disease demonstrates a significantly larger amount of perivascular connective tissue than nondiabetic subjects (26).

Epidemiologic studies (27) have shown that the incidence of congestive heart failure is increased in diabetic patients even when factors such as age, blood pressure, plasma cholesterol, weight and coronary artery disease are taken into account. Therefore, because of both its increased amount and different nature, collagen might be the plausible structural substrate of increased echodensity. These same changes did not result in substantial alterations in Doppler-derived indexes of left ventricular filling (28), although a relatively higher late peak velocity was observed. There was a large overlap in the two groups examined. However, the noninvasive evaluation of diastolic function poses some methodologic problems. In fact, all diastolic time-velocity measurements are strictly dependent on hemodynamic factors, such as preload or afterload and heart rate. The E/A ratio represents a global indicator of diastolic function. In our group of patients without clinical evidence of heart disease, this Doppler variable substantially overlapped in both study groups. This finding demonstrates the poor sensitivity of this Doppler index compared with integrated backscatter indexes, at least in patients without clinical symptoms.

Comparison with previous studies. Perez et al. (9) were the first to address the hypothesis that fibrosis or other myopathic changes could affect the acoustic properties of the myocardium in diabetic patients, signifying an occult cardiomyopathy. They found a reduction in cyclic variation from the myocardial wall of diabetic hearts compared with that in control hearts and attributed it to fibrosis or other occult cardiomyopathic changes in diabetic patients. They also found that these changes were more marked in the presence of

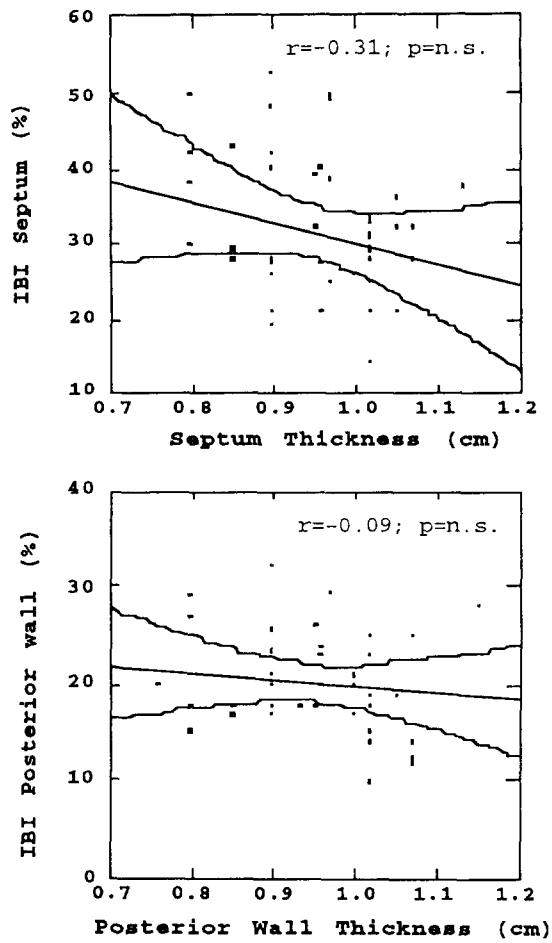


Figure 4. Top, Scatter plot showing the lack of correlation between the integrated backscatter index (IBI) and the diastolic thickness of the interventricular septum. **Bottom**, Scatter plot showing the lack of correlation between the integrated backscatter index and the diastolic thickness of the posterior wall.

noncardiac diabetic complications, such as neuropathy and retinopathy.

Our study differs from the pioneering observations of Perez et al. (9) in many respects, including patient selection and, most important, the indexes used for ultrasound tissue characterization. In patient selection, we excluded those with hypertension, which is frequently associated with diabetes but may itself cause a variation in ultrasound tissue characterization indexes (29,30). In the study of Perez et al. (9), patients with noncardiac complications of diabetes had more frequent hypertension, which may itself result in a blunting of the cyclic variation. Furthermore, although the overwhelming majority of patients in the study of Perez et al. (9) had normal conventional echocardiographic findings, this was not an inclusion criterion as it was in the present study. All of our patients had negative exercise stress test results, making the presence of concomitant coronary artery disease unlikely. Overall, our study patients were more strictly selected and more likely to represent the specific effects of diabetes on the heart, rather than those of associated diseases, such as hypertension or coronary artery disease.

Finally, and most important, we used a conceptually different approach to tissue characterization. The cyclic variation of backscatter used by Perez et al. (9) is related to contractile function but in a complex, nonlinear manner. The mechanisms of cyclic variation of backscatter are not completely understood, but they are likely to reflect physiologic rather than directly biochemical determinants of myocardium. We used, instead, the absolute backscatter amplitude, evaluated at end-diastole, a variable more closely related to the structural histologic composition of the wall. In particular, the amplitude of echodensity is directly and linearly related to the biochemically or histologically assessed collagen content of tissue (15,16). Taken together, the data of Perez et al. (9) and our own results emphasize the potential of ultrasound tissue characterization in detecting subtle physiologic and structural abnormalities at a preclinical stage, when conventional echocardiographic markers are still in the normal range.

Increased myocardial echodensity in insulin-dependent diabetic patients possibly represents a preclinical alteration, conceivably related to the myocardial collagen content increase, which does not necessarily indicate an actual disease but may be considered an early marker of the histopathologic findings of diabetic cardiomyopathy. Furthermore, these augmented ultrasound backscatter indexes probably predate the subclinical alterations in diastolic filling and systolic function, previously described by different techniques, such as systolic time intervals, radionuclide ventriculography and Doppler echocardiography (31–37).

Increased myocardial backscatter: a sign of impending diabetic cardiomyopathy. A hypothesis for a biologic scenario underlying our findings might predict, at the myocardial level, a situation similar to "impending" diabetic nephropathy. In the diabetic kidney, a widening of the glomerular basement membrane has been described (22), a clinical nephropathy in the absence of any detectable nephropathy, which may be demonstrated only after 15 to 20 years of diabetes. Diabetologists define "incipient" nephropathy as a clinical condition characterized by albuminuria that goes clinically undetected by current methodologies. The microalbuminuric patient is "normal" and normotensive according to classic clinical criteria but has a very high risk of developing clinical nephropathy. Similarly, type I insulin-dependent diabetes might have a preclinical phase during which an occult myocardial "alteration" develops (either by microangiopathy or by nonenzymatic glycation), conceivably due to metabolic disease itself. This may lead very early on to inappropriate connective tissue deposition, detectable noninvasively only by sophisticated methodology, such as ultrasound backscatter. The predictive power for the development of clinical diabetic cardiomyopathy of these radiofrequency indexes of cardiac damage must be confirmed. Follow-up studies addressing this crucial issue are currently in progress.

Limitations of the study. Some limitations of this study should be mentioned. 1) No independent histologic or other

direct evidence of myocardial abnormalities was available for the study subjects. This problem was unavoidable because myocardial biopsies would not have been ethically justified in these patients, who were clinically healthy from a cardiovascular viewpoint. 2) The analysis of standard indexes, derived from two-dimensional and Doppler echocardiography of global systolic and diastolic function, did not permit clear differentiation between the study groups. This was more clearly achieved, both as group and individual patient analysis, when ultrasound tissue characterization indexes were considered. Subtler levels of impairment in these patients might have been detected with more sophisticated assessment of left ventricular function, such as evaluation of contractility using load-independent measures.

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